A Volumetric Analysis (Acid-Base Titration) of the Acidity of Grape Must

In this week’s laboratory, we will titrate a Grape Must with Sodium Hydroxide to determine the Must’s Titratable Acidity. This procedure will include a standardization of the Sodium Hydroxide solution against the Primary Standard Potassium Hydrogen Phthalate (KHP). To check our titration technique, we will also carry out a titration of a solid mixture containing a known percentage of KHP.

Although titration is one of the best known and useful of the classical methods of quantitative analysis, Volumetric Analyses such as Volumetric Titrimetry were late in being developed because accurate methods for measuring volume are difficult to come by.

According to Francis Holme’s book on bleaching, the “value” of pearl ashes was measured in 1756 by noting the number of teaspoonfuls of dilute nitric acid which had to be added before effervescence ceased. This was the first clear instance of using a volumetric approach to chemical analysis ...

The Development of Modern Chemistry
by Aaron J. Ihde

As implied, Volumetric Titrimetry involves measuring the volume of a solution of known concentration (titrant) that is needed to completely react with an analyte; in the above example the number of teaspoons of Nitric Acid added to the analyte Pearl Ash is measured. This volume measurement allows us to determine the amount of analyte present. It was not until 1806 that F.A.H. Descroizilles introduced the Burette as an instrument for delivering accurately measured volumes of titrant. These early burettes, which had a tendency to leak, were improved upon by Karl Friedrich Mohr and the Mohr Burette served as the standard until replaced by instruments with ground glass and Teflon stopcocks.

In this lab we will perform two different volumetric titrations involving Acid-Base neutralization reactions. The first will be to determine the amount of the weak acid KHP in a solid mixture. The second will be to determine the amount of acid in Grape Must.

KHP, or the monopotassium salt of Phthalic Acid, otherwise known as Potassium Hydrogen Phthalate, is a weak acid that is often used as a Primary Standard to Standardize basic titrants. Primary Standards must be
available in high purity, be stable during dry storage and when dissolved in Water, and be amenable to drying (i.e., they won’t decompose during heating or quickly reabsorb Water from the atmosphere after drying). KHP meets all of these criteria. The general formula for this salt is KHC₈H₄O₄.

The Structure of KHP (MW 204.22 g/mol)

When dissolved in Water, the salt gives the Biphthalate Ion:

\[ \text{KHC}_8\text{H}_4\text{O}_4^{(aq)} \rightarrow \text{K}^+^{(aq)} + \text{HC}_8\text{H}_4\text{O}_4^{-}(aq) \]  (Eq. 1)

It is the Biphthalate Ion that is titrated with the Hydroxide titrant:

\[ \text{HC}_8\text{H}_4\text{O}_4^{-}(aq) + \text{OH}^{-}(aq) \rightarrow \text{C}_8\text{H}_4\text{O}_4^{2-}(aq) + \text{H}_2\text{O} \]  (Eq. 2)

A change in the color of the Acid-Base Indicator Phenolphthalein, clear in its acidic form and pink in its basic form, added to the analyte solution, will determine the Endpoint of the titration. If the indicator has been chosen well, the Endpoint will coincide with the Equivalence Point of the titration:

\[ \text{Equivalents Acid} = \text{Equivalents Base} \]  (Eq. 3)

In our case, this means:

\[ \# \text{ moles KHP} = \# \text{ moles OH}^- \]  (Eq. 4)

To ensure that an Acid-Base indicator’s Endpoint coincides with the titration’s Equivalence Point, it is required that the pKₐ of the indicator be roughly equivalent to the pH of the analyte solution at the equivalence point. The pH ranges for the color change of some commonly used Acid-Base Indicators are listed in the Appendix. Note that Phenolphthalein has a pKₐ of about 9. This is a typical equivalence point pH for many weak organic acids such as those found in Musts.

KHP will serve as both the analyte in a mixture of unknown composition and the Primary Standard against which our Sodium Hydroxide titrant solution will be Standardized. In this second role, the concentration of the titrant can be determined by titrating a known mass of the KHP Primary Standard to the Phenolphthalein Endpoint. At this point,

\[ [\text{NaOH}] = \frac{\left( \frac{\text{mass KHP}}{\text{MW of KHP}} \right)}{\text{Vol Titrant [L]}} \]  (Eq. 5)
Now to the main purpose of the lab, the analysis of Grape Must.

When grapes are picked from the vines and crushed, the resulting liquid is called a Grape Must; from the Latin vinum mustum or young wine. This Must contains several organic acids as well as inorganic compounds, polyphenols, and nitrogen- and sulfur-containing compounds. It also contains pulp, skins, stems and seeds, collectively known as pomace. The Must is ultimately fermented into wine. Red grape Must is sent directly to fermentation tanks whereas white grape Must is sent to a press where the juice is separated from the skins before fermentation.

The acids present in Must, Tartaric Acid, Malic Acid, Lactic Acid, Acetic Acid, Citric Acid and Succinic Acid, are all relatively weak organic acids. Although the concentrations of these acids are low, these acids are significant constituents in determining the sensory properties of wines; tartness in particular. Too little acid and the wine tastes flat. Too much acid and the wine will assault your taste buds. When acids are properly countered by other ingredients in wine (alcohol, sugars, etc.), the wine is said to be "In-Balance." Indirectly, acid content affects pH, color, stability, and the product's shelf life.

Tartaric and Malic Acids are the principal organic acids in grapes. Tartaric acid (H₂Tart) and its salts, Bitartrate (HTart⁻) and Tartrate (Tart²⁻), are virtually resistant to respiratory oxidation in grapes and to bacterial degradation in wines. They are also the strongest of the organic acids present in grapes. They make up half or more of the total acidity of Musts and wines.

The Titratable Acidity measures the free Hydrogen Ion concentration in the Must as well as the concentrations any undissociated acids able to be neutralized by base; i.e., the wine's total acidity. The titratable acid content of grape Musts or wines is readily determined by titration.
with a strong base (Sodium Hydroxide) and is commonly reported only in terms of Tartaric Acid; i.e., assuming all the various acids exist as Tartaric Acid.

$$H_2\text{Tart}^{(aq)} + 2 \text{OH}^{-}(aq) \rightarrow \text{Tart}_2^{2-(aq)} + 2 \text{H}_2\text{O}$$

(Eq. 6)

The Titratable Acidity is then reported alternatively in units of grams/Liter:

$$\text{Titratable Acidity} = \frac{\text{# grams Tartaric Acid}}{\text{Liters Solution}}$$

(Eq. 7)

or as a "percentage":

$$\text{Titratable Acidity} = \frac{\text{# grams Tartaric Acid}}{\text{mL Solution}} \times 100 \%$$

(Eq. 8)

(Note, these definitions differ by a factor of 10.)

The Tartaric Acid content of Grape Must typically ranges from 0.2 – 1 %. In most grape wines, the range is:

<table>
<thead>
<tr>
<th>Wine Style</th>
<th>Rec. Acidity Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry White Wine</td>
<td>0.65 - 0.75 %</td>
</tr>
<tr>
<td>Sweet White Wine</td>
<td>0.70 - 0.85 %</td>
</tr>
<tr>
<td>Dry Red Wine</td>
<td>0.60 - 0.70 %</td>
</tr>
<tr>
<td>Sweet Red Wine</td>
<td>0.65 - 0.80 %</td>
</tr>
<tr>
<td>Sherry Grape Wines</td>
<td>0.50 - 0.60 %</td>
</tr>
<tr>
<td>Non-Grape White Wines</td>
<td>0.55 - 0.65 %</td>
</tr>
<tr>
<td>Non-Grape Red Wines</td>
<td>0.50 - 0.60 %</td>
</tr>
</tbody>
</table>

The determination of titratable acidity is of interest when studying a wine’s maturity, considering spoilage and stability, and making an acid-base balance. Also, in order to determine the proper amount of Sulfur Dioxide, a commonly used sanitizing agent and antioxidant, to add to a Must, wine producers need to know its titratable acidity.

When titrating Musts or wines, several interferences and sources of error need to be considered. Phenolic compounds and amino acids have the ability to behave as acids and bases; they are amphoteric substances. Fortunately, the interference of these compounds near the Endpoint is small. In addition, Sulfur Dioxide, sugars and other non-acid compounds can introduce errors, however these errors are also usually small. Dissolved Carbon Dioxide can introduce substantial error if it is not removed by boiling the Water used to dilute the sample wine or Must or by the application of a vacuum. Perhaps the largest source of error is the accurate detection of the Endpoint, particularly in red wines, where the inherent color of the wine may obscure the Endpoint color change.

Thus, we will begin by preparing and then Standardizing a Sodium Hydroxide solution. We will then use this titrant to titrate a KHP solution prepared from a solid mixture of known composition. This will allow us to independently verify the composition of the mixture and to confirm our ability to conduct an accurate Acid-Base titration. Finally, we will use the NaOH titrant to titrate a Grape Must, the results of which will allow us to determine the Must’s Titratable Acidity.
Pre-Lab Calculations

1. A 0.6008g sample of pure KHP was dissolved in 50 mL of Water and titrated to a Phenolphthalein Endpoint using a Sodium Hydroxide titrant. The starting burette volume was 0.22 mL. The ending burette volume was 29.35 mL. Calculate the concentration of the NaOH titrant.

2. A 1.3986g sample of an unknown powder containing KHP was dissolved in 50 mL of Water and titrated to a Phenolphthalein Endpoint using the above NaOH titrant. The starting burette volume was 0.01 mL and the ending burette volume was 35.65 mL. Calculate the % KHP in the sample.

3. Assume a Must is 0.65% in Titratable Acidity, calculate the volume of Must that will require about 20 mL of the above NaOH titrant to reach the Phenolphthalein Endpoint.
Procedure

Preparation of the NaOH Titrant

Boil 1 L of Deionized Water for about 5 minutes to remove dissolved Carbon Dioxide. Allow the Water to cool covered by a watch glass. Transfer while warm to a 1 L bottle. (Never store strong bases in glass containers as they will react with the glass, changing the concentration of the base and etching the container. Transfer between 7 and 8 mL of a 50% Sodium Hydroxide solution to the bottle of Water using a 10 mL graduated cylinder. Mix thoroughly and keep capped. Many students may find that they obtain more consistent results if they shake the bottle vigorously before each time they refill their burette.

Standardization of the NaOH Titrant

Dry 4-5g of Primary Standard KHP in a weighing bottle at 110°C for at least one hour. Cool for 30 minutes in a desiccator.

Weigh, to the nearest 0.1 mg, 3 ~0.6g samples of the dry KHP into separate 250 mL Erlenmeyer flasks and dissolve in 50 mL distilled water. Warm the solutions to promote dissolution of the KHP if needed.

Prepare the buret for titration by rinsing several times with small portions of the ~0.1 M NaOH solution. Fill the buret and adjust the level to be near the zero mark. Record the initial volume to the nearest 0.05 mL. Add 2-3 drops of Phenolphthalein Indicator to the first sample of standard KHP and titrate, adding the titrant no faster than 0.5 mL/second. Swirl the flask constantly. Titrate until the faint pink color remains for at least 20 seconds while continuing to swirl the flask. Record the volume of the buret. As you approach the Endpoint, use a squeeze bottle of DI Water to wash any stray droplets of titrant down into the flask. Titrate dropwise in the vicinity of the Endpoint and split drops at the end to obtain a very sharp Endpoint. (Your laboratory instructor will demonstrate how to split drops from a burette.) Repeat the titration procedure for the remaining KHP samples. [Note: Getting the Endpoint right the first time is not easy. You may wish to do an initial crude titration to get an estimate of where the Endpoint will occur. Then perform three careful titrations.]

Calculate the molarity of the NaOH solution for each trial. Calculate the average and standard deviation of these values. Use a Q-Test with a 90% confidence level as the criterion for rejecting suspected outliers. Check with your TA before moving on to the next part of the lab.

 Determination of the Amount of KHP in an Known

Dry the “known” sample at 110°C for at least one hour. Cool it 30 minutes in a desiccator. The powder contains between 20% and 70% KHP by mass.

Weigh out three portions of dried sample, ~1.4g each, into separate 250 mL Erlenmeyer flasks. Dissolve each sample with 50 mL DI Water; warming the samples if needed. Add 2-3 drops indicator.
Titr

ate each sample with your Standardized NaOH solution.

Calculate and report the % KHP by mass in the sample for each of your replicate titrations. Calculate the average and standard deviation of these values. Again, use the Q-Test if needed to reject suspect data. (Ideally, the standard deviation in the % KHP for three trials should be 0.2% or less. If your results are not close to 0.2% after three trials, you might want to perform a few more trials to improve your results.

**Determination of the Titratable Acidity of Grape Must**

Obtain an unknown grape Must sample from the instructor. Pipet one 10 mL aliquot into clean 250 mL Erlenmeyer flask, add about 50 mL boiling DI Water and ~10 drops phenolphthalein indicator. Note: for red grape Musts, the amount of indicator necessary to adequately see the endpoint color change might need to be increased. Titrate with your Standardized NaOH to the Endpoint. Repeat this procedure twice more.

Determine the amount of Titratable Acid in the grape Must for each trial. Calculate the average and standard deviation of these values. Use the Q-Test to reject data if needed.
## Appendix - Acid-Base Indicators

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Range</th>
<th>Low pH Color</th>
<th>High pH Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymol Blue</td>
<td>1.2 – 2.8</td>
<td>Red</td>
<td>Orange</td>
</tr>
<tr>
<td>Methyl Orange</td>
<td>3.1 – 4.4</td>
<td>Red</td>
<td>Yellow</td>
</tr>
<tr>
<td>Congo Red</td>
<td>3.0 – 5.0</td>
<td>Purple</td>
<td>Red</td>
</tr>
<tr>
<td>Methyl Red</td>
<td>4.8 – 6.0</td>
<td>Red</td>
<td>Yellow</td>
</tr>
<tr>
<td>Bromocresol Purple</td>
<td>5.2 – 6.8</td>
<td>Yellow</td>
<td>Purple</td>
</tr>
<tr>
<td>Bromthymol Blue</td>
<td>6.0 – 7.6</td>
<td>Yellow</td>
<td>Blue</td>
</tr>
<tr>
<td>Cresol Red</td>
<td>7.2 – 8.8</td>
<td>Orange</td>
<td>Red</td>
</tr>
<tr>
<td>Thymol Blue</td>
<td>8.0 – 9.6</td>
<td>Yellow</td>
<td>Blue</td>
</tr>
<tr>
<td>Phenolphthalein</td>
<td>8.0 – 9.6</td>
<td>Clear</td>
<td>Pink</td>
</tr>
<tr>
<td>Alizarin Yellow</td>
<td>10.1 – 12.0</td>
<td>Red</td>
<td>Purple</td>
</tr>
</tbody>
</table>